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THE RED CELL AND PLASMA VOLUMES OF THE RAT AND OF ITS INDIVIDUAL TISSUES AND ORGANS DURING ACCLIMATION TO COLD

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ABSTRACT

The red cell and plasma volumes of the total rat and of its individual tissues and organs have been determined for animals exposed to 5^{o} C for four hours, 24 hours, two weeks, and six weeks. In addition, the tissue hematocrits have been determined. These values have been compared to those of rats kept at 24^{o} C. Fe^{59} - labeled erythrocytes and I^{131} albumin were given intravenously and allowed to mix; the rats were then frozen in liquid nitrogen. The organs and tissues were removed in the frozen state, assayed for radioactivity, and blood cell and plasma volumes were calculated on a unit weight basis.

Significant changes in blood cell and plasma volumes were observed for the total rat and for many of the individual organs. There was a significant increase in the red cell content of the total rat within 24 hours of cold exposure. After six weeks, the total blood volume was increased by approximately 20% over the control level, and the increase in erythrocyte volume was slightly more than the increase in plasma volume. The hematocrit of heart blood was 44.8 after six weeks exposure as compared to 41.5 for controls. In general, it can be said that the somatic parts of the body showed increases in blood volume whereas the visceral parts had decreased volumes.

THE RED CELL AND PLASMA VOLUMES OF THE RAT AND OF ITS INDIVIDUAL TISSUES AND ORGANS DURING ACCLIMATION TO COLD*

Several studies have reported circulatory changes as a part of an animal's adjustment during acute or chronic exposure to cold. (See Carlson (1954) and Bass and Henschel (1956) for reviews.) Only a few of these studies, however, have been concerned with blood volume changes, and none have concerned the blood volume of individual organs and tissues. Furthermore, the studies which have been reported for the changes in total body blood following cold exposure are not in common agreement. Bazett, et al. (1940) and Scott, et al. (1940) reported a decrease in the blood of man upon exposure to cold. Bazett, et al. (1940) found that the decrease was reflected in both the circulating hemoglobin and in the plasma. Adolph and Molnar (1946) likewise noted a decrease in blood volume incident to cold exposure, but the blood increased in concentration, indicating a loss of plasma volume. They suggested that the vascular bed is greatly reduced by vasoconstriction and that the plasma volume is decreased to fit the bed. It was inferred that the observed escape of fluid into the urine was associated with the diminution of plasma volume. Similarly, Doupe, et al. (1957) reported from their plasma volume determinations of young men in Winnipeg that there was an increase in blood volume in summer and that the trend was reversed in fall and winter.

In contrast to the above observations, Brown reported that the blood volume of Eskimos increased in winter and decreased in summer. Elsner (1955), however, found no change in plasma volume of men who lived for 3 1/2 weeks in a tent near Fairbanks where the ambient temperature was from -45° to $+34^{\circ}$ F. Similarly, Overman and Feldmen (1947) found that the plasma volume of monkeys was not significantly different in summer than in winter.

One of the more recent and comprehensive studies relating to body fluid adjustments in experimental animals during acclimation to cold was made by Deb and Hart (1956). They found that rats after five weeks at 6° C evidenced a 22% increase in blood volume over animals kept at 30° C.

Since the results of the above studies are contradictory, it was deemed desirable to use experimental animals in an extensive study of blood volume following cold exposure. Furthermore, since no organ blood volume measurements have been reported for

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animals subjected to cold, it seemed desirable to expand the study to include a determination of the blood content of multiple organs and tissues using methods developed in this laboratory (1956).

METHODS

The animals used were male rats of the Sprague-Dawley strain. Four experimental groups were subjected to 5° C for periods of four hours, 24 hours, two weeks, and six weeks. The controls were kept at 24° C. The experimental animals, individually caged, were placed in the cold room at a time which would allow them to weigh approximately 200 to 225 g at the end of the exposure period. Thirty or more rats were used for controls and for each period of cold exposure. Approximately one-third of the animals in each group was used for the determination of the plasma content of organs, one-third for the determination of red cell content, and the remainder for duplicate hematocrit determinations on blood withdrawn from the heart.

The hematocrits, determined by the method of Wintrobe (1946), were corrected for the 4% plasma trapped in the red cellpack. The methods employed for determining the red cell and plasma content of the tissues were the same as described in detail by Everett, et al. (1956). Fe^{59}-labeled erythrocytes from donor rats and I^{131} human serum albumin (Risa, Abbott) were used for the measurements of whole blood weights. At the end of the period of cold exposure and without removing the animals from the cold room, Fe^{59}- labeled erythrocytes with an activity of approximately 4×10^5 counts min/ml or 2 μc of I^{131} albumin were injected into the great saphenous vein.** The injection volume in both cases was 0.2 ml.

After allowing for mixing (three minutes for albumin and 15 minutes for the tagged cells), the rats were frozen in liquid nitrogen as previously described. The animals were then stored at -15° C for one or more days; they were dissected in the frozen state using instruments chilled with solid CO_2. The methods employed for dissecting and

*There was essentially no change in weight during the first week of cold exposure, and thereafter the increase was about 3.5 g per day.

**Both Fe^{59}-labeled cells and I^{131} albumin were injected into some of the rats of the six-week exposure group and a gamma ray spectrometer was employed to assay the tissue samples for I^{131} and for Fe^{59} content.

sampling of the tissues were the same as described previously. The respective organ and tissue samples were placed in glass shell vials, weighed and assayed in a well-type scintillation counter. Red cell, plasma, and total blood volumes were calculated by the following formulas:

$$\text{mg blood/g tissue} = \frac{\text{activity/g tissue}}{\text{activity/mg heart blood}}$$

$$\text{RCV } (\mu\text{l red cells/g tissue}) = \frac{\text{mg blood/g tissue}}{1.056^{**}} \text{ X Hct* of heart blood}$$

$$\text{Pv } (\mu\text{l plasma/g tissue}) = \frac{\text{mg blood/g tissue}}{1.056} \text{ X 1-Hct of heart blood}$$

Combining the values:

$$\text{total blood volume (TBV) } (\mu\text{l blood/g tissue}) = \text{RCV + Pv}$$

$$\text{tissue Hct} = \frac{\text{RCV}}{\text{TBV}}$$

RESULTS

The mean hematocrit values for heart blood of control rats and for animals exposed to cold appear in Table I. It is noted that the hematocrit was depressed in animals exposed for four hours and for 24 hours, but was considerably above the control value after two weeks and after six weeks exposure to 5° C.

The control values for the red cell volume, plasma volume, total blood volume, and for hematocrits of the multiple organs have been published (Everett, et al., 1956) and are not repeated here as individual values. The blood values for the organs and tissues of the rats exposed to cold for four hours, 24 hours, two weeks, and for six weeks are shown respectively in Tables II, III, IV, and V. It may be observed that the percentage deviation from control values is shown for the total blood volumes (Pv + RCV) and for the tissue hematocrits (Hct).

*Hematocrit.

**Specific gravity of rat

TABLE I

HEMATOCRIT VALUES OF HEART BLOOD

	No. of Animals	Average Hematocrit	% σm
Controls	18	41.5	0.13
4-hour cold exposure	9	40.6	0.84
24-hour cold exposure	21	40.5	1.09
2-week cold exposure	12	43.2	0.74
6-week cold exposure	9	44.8	1.00

TABLE II

BLOOD VALUES AFTER FOUR HOURS AT 5° C

	Fe^{59} Red Cell Measurements			I^{131} Albumin Measurements			Combined Data					
	No. Rats	Mean Blood mg/g	RCV l/g	M No. Rats	Mean Blood mg/g	PV l/g	Mean PV + RCV l/g	%Δ from Cont.	of Mean	m% of Mean	Hct	%Δ from Cont.
Total rat	9	53.6	20.6	9	64.5	36.3	56.9	+2.3	.9	1.7	36.2	-1.4
Adrenal	10	96.9	37.3	10	314	177	214	-10.1	7.5	3.5	17.4	-11.7
Bone	8	16.5	6.34	9	64.0	36.0	42.3	+4.4	2.0	4.8	15.0	+8.7
Cerebral hemisph.	10	25.2	9.69	9	41.1	23.1	32.8	+9.3	1.4	4.4	29.5	+1.0
Midbrain & thal.	8	27.2	10.5	8	33.7	19.0	29.5	-7.2	1.9	6.5	35.6	+2.0
Cerebellum	10	33.8	13.0	9	43.7	24.6	37.6	-0.5	1.7	4.6	34.6	+6.5
Pons & medulla	8	15.0	5.77	9	23.4	13.2	19	-15.9	.6	3.5	30.4	+4.8
Cardiac muscle	9	199	76.5	9	340	191	268	+2.3	10.2	3.8	28.5	+5.2
Hypophysis	6	98.1	37.7	5	142	79.9	118	+27.4	7.8	6.6	31.9	-11.4
Kidney	8	46.9	18.0	10	165	92.8	111	-13.3	4.6	4.2	16.2	-27.7
Liver	8	189	72.7	10	307	173	246	-8.9	10.6	4.3	29.6	-3.0
Lung	10	501	193	10	599	337	530	+2.1	13.2	2.5	36.4	+5.5
Seminal vesicle	7	21.3	8.19	7	25.1	14.1	22.3	-3.0	1.6	7.1	36.7	-2.4
Skeletal muscle	8	23.8	9.15	9	32.4	18.2	27.4	+6.2	.8	2.9	33.4	+3.1
Skin	7	20.1	7.73	10	26.8	15.1	22.8	+18.8	.6	2.8	33.9	-10.6
Small intestine	10	19.7	7.57	10	39.2	22.0	29.6	-12.7	2.1	7.0	25.6	-2.7
Spinal cord	8	26.1	10.0	9	38.3	21.5	31.5	+25.5	2.9	9.1	31.7	+13.6
Spleen	9	110	42.3	9	141	79.3	122	-28.2	5.2	4.3	34.7	-31.6
Sub. max. gland	9	66.8	25.7	7	82.8	46.6	72.3	-11.1	4.9	6.8	35.5	+14.1
Testis	10	11.4	4.38	9	20.3	11.4	15.8	+0.6	.9	6.0	27.7	+4.5
Thyroid	8	172	66.1	8	200	112	178	-1.7	16.7	9.4	37.1	-29.6

TABLE IV

BLOOD VALUES AFTER TWO WEEKS AT 5° C

	Fe^{59} Red Cell Measurements			I^{131} Measurements					Combined Data			
	No. Rats	Mean Blood mg/g	RCV µl/g	No. Rats	Mean Blood mg/g	PV µl/g	Mean PV + RCV µl/g	%Δ from Cont.	of Mean	m% of Mean	Hct	%Δ from Cont.
Total rat	12	60.8	24.9	10	75	40.3	65.2	+17.3	1.5	2.3	38.2	+4.1
Adrenal	15	118	48.3	11	300	161	209	-12.2	7.9	3.8	23.1	+17.3
Bone	15	17.0	6.95	11	70.2	37.8	44.8	+10.6	1.9	4.2	15.5	+12.3
Cerebral Hemisph.	15	25.5	10.4	11	39.8	21.4	31.8	+6.0	0.6	2.0	32.7	+11.6
Midbrain & thal.	14	29.3	12.0	11	43.9	23.6	35.6	+11.9	1.6	4.5	33.7	-3.4
Cerebellum	13	34.9	14.3	11	49.5	26.6	40.9	+8.2	1.4	3.5	35.0	+7.7
Pons & medulla	12	15.9	6.50	11	26.0	14.0	20.5	-9.3	0.7	3.3	31.7	+9.3
Cardiac muscle	15	188	76.9	11	297	160	237	-9.5	6.4	2.7	32.4	+19.6
Hypophysis	13	104	42.5	8	110	59.2	102	+10.2	8.7	8.5	41.7	+15.8
Kidney	14	58.8	24.1	11	197	106	130	+1.6	3.2	2.5	18.5	-17.4
Liver	11	208	85.1	10	347	187	272	+0.7	3.8	1.4	31.3	+2.6
Lung	14	512	209	8	662	356	565	+8.9	7.9	1.4	37.0	+7.2
Seminal vesicle	12	18.1	7.40	8	16.3	8.77	16.2	-29.6	1.0	6.3	45.7	+21.5
Skeletal muscle	12	25.2	10.3	10	37.4	20.1	30.4	+17.8	0.7	2.4	33.9	+4.6
Skin	13	21.8	8.92	11	27.7	14.9	23.8	+24.0	1.3	5.4	37.5	-1.1
Small intestine	14	18.0	7.36	11	46.3	24.9	32.3	-4.7	1.6	4.8	22.8	13.3
Spinal cord	13	26.4	10.8	10	29.2	15.7	26.5	+5.6	2.2	8.5	40.8	+46.2
Spleen	8	138	56.5	11	140	75.3	132	-22.4	5.1	3.9	42.8	-15.6
Sub. max. gland	15	62.3	25.5	10	75.2	40.4	65.9	-18.9	3.6	5.5	38.7	+24.4
Testis	15	14.1	5.77	11	23.2	12.5	18.[illegible]	+16.6	0.7	4.1	31.5	+18.9
Thyroid	12	198	81.0	8	232	125	206	+13.8	27.2	13.2	39.3	-25.4

large vessels throughout the body. For this reason, the cell dilution method alone tends to underestimate blood volume, and the plasma method alone overestimates blood volume. Furthermore, it is only by using the double method, as reported here, that tissue hematocrits can be obtained.

In addition, it is to be emphasized that, in order to provide for the most accurate assessment of plasma volume employing I^{131} albumin or comparable sized molecules, the mixing time should be kept to the minimum consistent with complete mixing. The three minutes employed here for I^{131} and 15 minutes for labeled cells were previously established by Everett, et al. (1956) to be appropriate for the rat. Extended periods lead to an escape of significant amounts of I^{131} albumin into the tissue spaces of some organs (Everett and Simmons, 1958).

It is known that cold acclimation is associated with a decrease in body fat (Page and Babineau, 1953), and that body fluid volumes are influenced by fat content (Deb and Hart, 1956). It was for these reasons that the rats used in this study were selected within the weight range of 180 to 225 g. Sprague-Dawley rats of this weight range have not accumulated sufficient body fat to significantly alter the validity of expressing the fluid volumes on a body weight basis. Limiting the final weight range of the animals as indicated meant that the two-week and six-week groups were comprised of slightly younger animals at the onset of the exposure period. It is questionable that this slight difference in age and weight at the onset of cold exposure would influence the results. It is deemed important, however, for the rats in each group to weigh essentially the same as the controls at the end of the exposure period.

It is clear from this study that there are significant changes in the blood cell and plasma volume of the rat incident to cold exposure. For the total rat, there is an increase in both erythrocyte and plasma content per unit weight of tissue. These findings are in line with those of Deb and Hart (1956), who reported that rats after five weeks' exposure to 6° C had an increased blood volume of 22%. Although these investigators used only the plasma dilution method for blood volume determinations, it is apparent that the increase in blood volume reflected an increase in both plasma and red cell volume since an increase in hematocrit accompanied the increase in blood volume.

The results reported here show an increase in hematocrit from 41.5 to 44.8 after six weeks' exposure to 5° C. This increase is of the same order of magnitude as that reported by Sutherland and Campbell (1956) and by Sutherland, et al. (1958) for the rabbit after eight weeks' exposure to 4° C. Hannon, et al. (1958) observed that the hematocrit of rats increased approximately 3% after four weeks exposure to 5° C. More recently

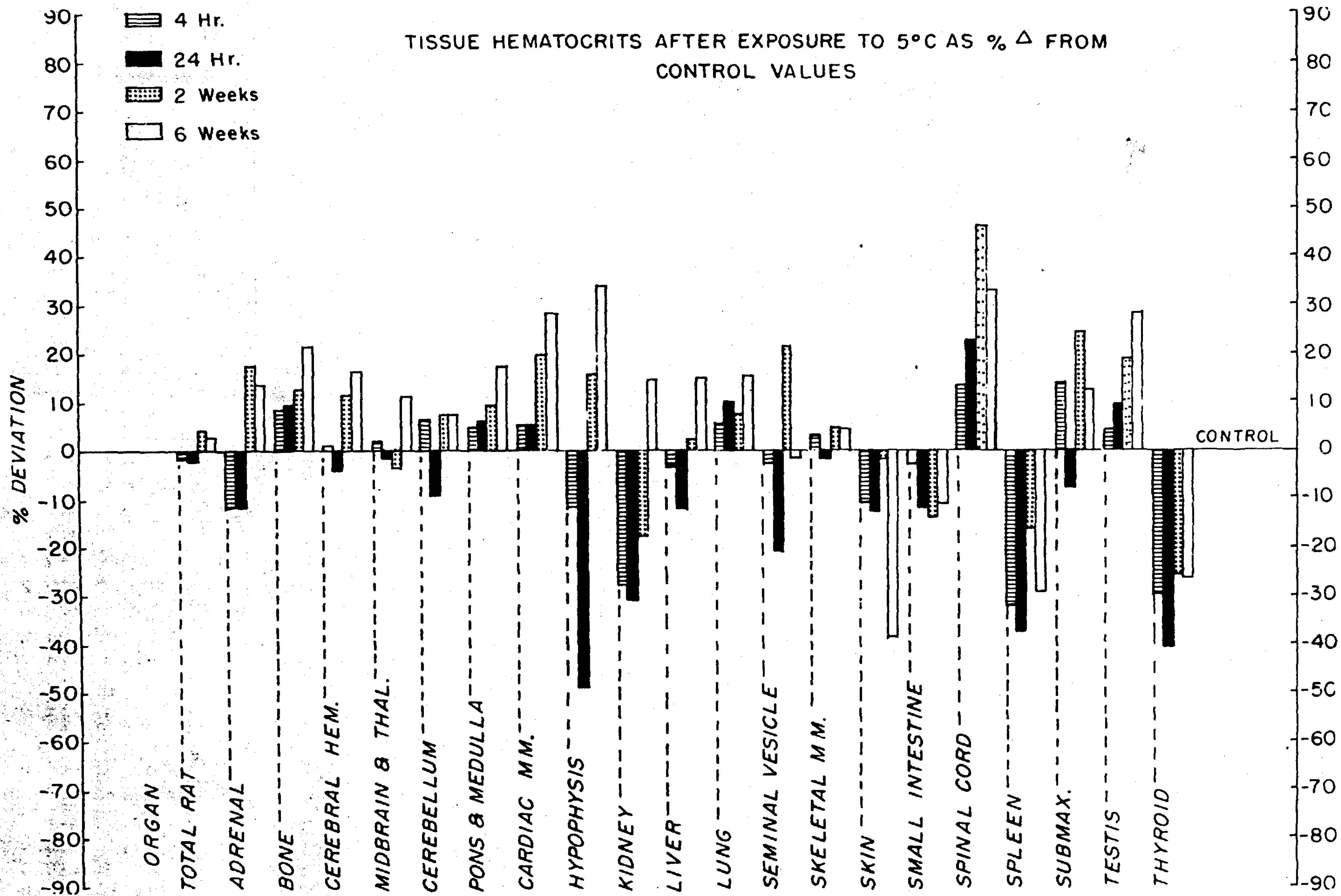
TISSUE HEMATOCRITS AFTER EXPOSURE TO 5°C AS % Δ FROM
CONTROL VALUES
4 Hr.
24 Hr.
2 Weeks
6 Weeks
% DEVIATION
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
CONTROL
ORGAN
TOTAL RAT
ADRENAL
BONE
CEREBRAL HEM.
MIDBRAIN & THAL.
CEREBELLUM
PONS & MEDULLA
CARDIAC MM.
HYPOPHYSIS
KIDNEY
LIVER
LUNG
SEMINAL VESICLE
SKELETAL MM.
SKIN
SMALL INTESTINE
SPINAL CORD
SPLEEN
SUBMAX.
TESTIS
THYROID

FIGURE 4

TABLE V

BLOOD VALUES AFTER SIX WEEKS AT 5° C

	Fe^{59} Red Cell Measurements			I^{131} Albumin Measurements					Combined Data			
	No. Rats	Mean Blood mg/g	RCV µl/g	No. Rats	Mean Blood mg/g	PV µl/g	Mean PV + RCV µl/g	% Δ from Cont.	of Mean	m% of Mean	Hct	% Δ from Cont.
Total rat	20	59.1	25.1	26	49.4	41.5	66.6	+19.8			37.7	+2.7
Adrenal	11	114	48.4	21	321	168	216	-9.2	8.4	3.9	22.4	+13.7
Bone	11	17.7	7.51	19	71.0	37.1	44.6	+10.1	1.5	3.3	16.8	+21.7
Cerebral hemisph.	11	22.8	9.67	21	35.6	18.6	28.3	-5.7	.5	1.8	34.2	+16.7
Midbrain & thal.	13	32.4	13.7	17	41.6	21.7	35.4	+11.3	1.4	3.9	38.7	+10.9
Cerebellum	13	29.1	12.3	20	43.9	22.9	35.2	-6.9	1.1	3.0	34.9	+7.4
Pons & medulla	11	14.6	6.19	15	23.0	12.0	18.2	-19.5	0.6	3.4	34.0	+17.2
Cardiac Muscle	10	184	78.1	20	282	147	225	-14.1	4.2	1.9	34.7	+28.0
Hypophysis	2	148	62.8	8	129	67.4	130	+40.4	19.9	15.3	48.3	+34.2
Kidney	12	82.9	35.2	20	196	102	137	+7.0	3.8	2.8	25.7	+14.7
Liver	12	210	89.1	19	315	165	254	-5.9	5.1	2.0	35.1	+15.1
Lung	13	543	230	22	662	346	576	+11.0	9.2	1.6	39.9	+15.7
Seminal vesicle	7	14.4	6.11	9	19.8	10.4	16.5	-28.3	1.4	8.3	37.0	-1.6
Skeletal muscle	11	24.1	10.2	18	38.1	19.9	30.1	+16.7	0.7	2.3	33.9	+4.6
Skin	13	16.0	6.79	20	28.8	15.1	21.9	+14.1	0.8	3.5	23.3	-18.2
Small intestine	11	18.5	7.85	21	49.2	25.7	33.6	-0.9	1.6	4.9	23.4	-11.0
Spinal cord	11	29.3	12.4	16	40.2	21.0	33.4	+33.1	2.3	7.0	37.1	+33.0
Spleen	11	90.4	38.4	18	131	68.5	107	-37.1	3.5	3.3	35.9	-29.2
Sub. max. gland	13	53.6	22.7	20	80.8	42.2	64.9	-20.2	1.9	3.0	35.0	+12.5
Testis	13	18.9	8.02	19	29.9	15.6	23.6	+50.3	0.8	3.4	34.0	+28.3
Thyroid	6	171	72.4	13	217	113	185.	+2.2	12.0	6.5	39.1	-25.8

Watanabe (1958) has reported the hematocrit of human subjects in Japan to vary monocyclically throughout the year, rising in winter and falling in summer.

Even though there was an over-all increase in blood volume of approximately 20% in rats during the six-week period of cold exposure, it is apparent that the increase was not uniform for all organs. It is of interest that some organs evidenced an increase in both red cells and plasma, others an increase in only one of these, and still others a decrease in one or both. It is important to note that the change in blood cell or plasma content of an organ during the early periods of exposure did not always indicate what the magnitude or direction of the change would be by the end of the adaptation period.

Although the present study is not concerned directly with the mechanisms which are responsible for the particular changes in blood content during cold exposure, it would seem appropriate to make a few general comments relative to certain specific changes. Many of the increases in tissue red cell content unquestionably relate to the increased heat production. The associated increase in metabolic rate and need for more oxygen by the tissues concerned would provide the stimulus for increased red cell production and, thus, lead to increases in tissue red cell content. Skeletal muscle would be the prime example with an increase in red cell content of approximately 25%. With respect to the significant increase in blood volume of skeletal muscle, it is pertinent that Heroux (1956) detected an increased number of capillaries in the leg muscles of the cold-acclimated rat.

The great increase in the red cell content of bone would seem to be related to the increase in red cell production in the bone marrow. Within this same context, the decrease of 50% to 60% in red cell content of the spleen would be in accord with the concept that the spleen does indeed serve for storage of erythrocytes, and an adequate stimulus was provided for their release.

The pronounced increase in erythrocyte content of the lung might be expected to provide for the additional gaseous exchanges associated with an increase in metabolic rate.

The spinal cord was the only division of the central nervous system which evidenced a change of considerable magnitude, and this was an increase in red cell content of approximately 80% within six weeks. This probably relates to the increased skeletal muscle activity which would be concerned with increased heat production.

Attention should be directed to a number of organs which had significant changes in blood content at the early intervals of cold exposure, and by six weeks the blood content per unit weight was not significantly different from control values. These include adrenal, kidney, liver (red cell content), and small intestine. Changes of this type probably reflect a temporary redistribution of blood concomitant with the disturbed hemostatic mechanisms. It could be that these changes relate to the hypertrophy of these organs during cold acclimation which has been reported for the rat by Heroux and Gridgeman (1957). Page and Babineau (1953) have also reported a hypertrophy of the liver and kidneys of rats during prolonged exposure to cold.

The thyroid, which might be expected to show increases in blood volume since thyroxine production is increased during cold exposure (Knigge, 1957), did, in fact evidence significant increases in plasma volume. Although the red cell measurements indicated a decrease in erythrocyte content, the decrease was not significant at the 0.05 level. The thyroid, because of its very small size, presents some difficulties in recovering intact from the frozen animals, as well as in weighing and assaying to the desirable level of accuracy. The hypophysis is another organ which presents comparable difficulties.

An additional comment which might be made is that, in general, the somatic parts of the body (see bone, skeletal muscle, and spinal cord) show increases in blood content during cold acclimation whereas many visceral parts show decreases (see adrenal, liver, seminal vesicle, small intestine, spleen, and submaxillary gland).

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